486. (Twice Amended) A method [of detecting] for determining whether one or more non-viral target [non-viral] species may be present in a sample, said method comprising the steps of:

a) contacting said sample with hybridization assay means for detecting [if] the presence of a nucleic acid variable region characteristic of nucleic acid of said one or more target species [may be present in said sample], wherein said means distinguishes [a] said [nucleic acid] variable region [characteristic of said one or more target species] from nucleic acid of at least one non-target species, wherein said variable region is present in an rRNA sequence, or a DNA sequence encoding for said rRNA sequence, in a location corresponding to a target region selected from the group consisting of:

bases 65-108 of *E. coli* 5S rRNA or the encoding DNA;
bases 60-105 of *E. coli* 16S rRNA or the encoding DNA;
bases 120-150 of *E. coli* 16S rRNA or the encoding DNA;
bases 170-230 of *E. coli* 16S rRNA or the encoding DNA;
bases 405-490 of *E. coli* 16S rRNA or the encoding DNA;
bases 600-675 of *E. coli* 16S rRNA or the encoding DNA;
bases 705-735 of *E. coli* 16S rRNA or the encoding DNA;
bases 820-870 of *E. coli* 16S rRNA or the encoding DNA;
bases 980-1060 of *E. coli* 16S rRNA or the encoding DNA;
bases 1125-1155 of *E. coli* 16S rRNA or the encoding DNA;
bases 1250-1290 of *E. coli* 16S rRNA or the encoding DNA;
bases 270-405 of *E. coli* 23S rRNA or the encoding DNA;
bases 535-575 of *E. coli* 23S rRNA or the encoding DNA;
bases 1150-1200 of *E. coli* 23S rRNA or the encoding DNA;
bases 1440-1600 of *E. coli* 23S rRNA or the encoding DNA;
bases 1710-1750 of *E. coli* 23S rRNA or the encoding DNA; and
bases 2190-2330 of *E. coli* 23S rRNA or the encoding DNA[;],

provided that if said target region is present in a location corresponding to bases 1250-1290 of *E. coli* 16S rRNA, or the encoding DNA, then said one or more target species is *Mycoplasma pneumoniae*; and

b) determining [if] whether said means [detects] has detected the presence of said [nucleic acid] variable region [characteristic of said one or more target species] as an indication [of] that at least one member of said one or more target species [may be] is present in said sample.

487. (Twice Amended) A method [of detecting] for determining whether one or more non-viral target [non-viral] species may be present in a sample, said method comprising the steps of:

a) contacting said sample with an oligonucleotide probe which distinguishes between nucleic acid of said one or more target species from nucleic acid of at least one non-target species, wherein a duplex formed between said oligonucleotide probe and a variable region present in [said] nucleic acid of said one or more target species has a higher T_m than a duplex formed between said oligonucleotide probe and said variable region present in [said] nucleic acid of said at least one non-target species, wherein said variable region is present in an rRNA sequence, or a DNA sequence encoding for said rRNA sequence, in a location corresponding to a target region selected from the group consisting of:

bases 65-108 of *E. coli* 5S rRNA or the encoding DNA;
bases 60-105 of *E. coli* 16S rRNA or the encoding DNA;
bases 120-150 of *E. coli* 16S rRNA or the encoding DNA;
bases 170-230 of *E. coli* 16S rRNA or the encoding DNA;
bases 405-490 of *E. coli* 16S rRNA or the encoding DNA;
bases 600-675 of *E. coli* 16S rRNA or the encoding DNA;
bases 705-735 of *E. coli* 16S rRNA or the encoding DNA;
bases 820-870 of *E. coli* 16S rRNA or the encoding DNA;
bases 980-1060 of *E. coli* 16S rRNA or the encoding DNA;

bases 1125-1155 of *E. coli* 16S rRNA or the encoding DNA;
bases 1250-1290 of *E. coli* 16S rRNA or the encoding DNA;
bases 270-405 of *E. coli* 23S rRNA or the encoding DNA;
bases 535-575 of *E. coli* 23S rRNA or the encoding DNA;
bases 1150-1200 of *E. coli* 23S rRNA or the encoding DNA;
bases 1440-1600 of *E. coli* 23S rRNA or the encoding DNA;
bases 1710-1750 of *E. coli* 23S rRNA or the encoding DNA; and
bases 2190-2330 of *E. coli* 23S rRNA or the encoding DNA[;],

provided that if said target region is present in a location corresponding to bases 1250-1290 of *E. coli* 16S rRNA, or the encoding DNA, then said one or more target species is *Mycoplasma pneumoniae*; and

b) [detecting the presence of] determining whether a nucleic acid complex comprising said oligonucleotide probe has formed under conditions of high stringency as an indication that at least one member of said one or more target species [may be] is present in said sample, wherein [under said conditions] said oligonucleotide probe does not form a detectable duplex with [said] nucleic acid of said at least one non-target species under said conditions.

---

488. (Twice Amended) A method [of detecting] for determining whether one or more non-viral target [non-viral] species may be present in a sample, said method comprising the steps of:

a) contacting said sample with hybridization assay means for detecting [if] the presence of a nucleic acid variable region characteristic of nucleic acid of said one or more target species [may be present in said sample], wherein said means distinguishes [a nucleic acid] said variable region [characteristic of said one or more target species] from nucleic acid of at least one non-target species belonging to the same genus as said one or more target species, wherein said variable region is present in an rRNA sequence, or a DNA sequence encoding for said rRNA sequence, in a location corresponding to a target region selected from the group consisting of:

bases 65-108 of *E. coli* 5S rRNA or the encoding DNA;
bases 60-105 of *E. coli* 16S rRNA or the encoding DNA;
bases 120-150 of *E. coli* 16S rRNA or the encoding DNA;
bases 170-230 of *E. coli* 16S rRNA or the encoding DNA;
bases 405-490 of *E. coli* 16S rRNA or the encoding DNA;
bases 600-675 of *E. coli* 16S rRNA or the encoding DNA;
bases 705-735 of *E. coli* 16S rRNA or the encoding DNA;
bases 820-870 of *E. coli* 16S rRNA or the encoding DNA;
bases 980-1060 of *E. coli* 16S rRNA or the encoding DNA;
bases 1125-1155 of *E. coli* 16S rRNA or the encoding DNA;
bases 1250-1290 of *E. coli* 16S rRNA or the encoding DNA;
bases 270-405 of *E. coli* 23S rRNA or the encoding DNA;
bases 535-575 of *E. coli* 23S rRNA or the encoding DNA;
bases 1150-1200 of *E. coli* 23S rRNA or the encoding DNA;
bases 1440-1600 of *E. coli* 23S rRNA or the encoding DNA;
bases 1710-1750 of *E. coli* 23S rRNA or the encoding DNA; and
bases 2190-2330 of *E. coli* 23S rRNA or the encoding DNA[;],

provided that if said target region is present in a location corresponding to bases 1250-1290 of *E. coli* 16S rRNA, or the encoding DNA, then said one or more target species is *Mycoplasma pneumoniae*; and

b) determining [if] whether said means [detects] has detected the presence of said [nucleic acid] variable region [characteristic of said one or more target species] as an indication that at least one member of said one or more target species [may be] is present in said sample.

489. (Twice Amended) A method [of detecting] for determining whether one or more non-viral target [non-viral] species may be present in a sample, said method comprising the steps of:

a) contacting said sample with an oligonucleotide probe which distinguishes between nucleic acid of said one or more target species from nucleic acid of at least one non-target species belonging to the same genus as said one or more target species, wherein a duplex formed between said oligonucleotide probe and a variable region present in [said] nucleic acid of said one or more target species has a higher T_m than a duplex formed between said oligonucleotide probe and said variable region present in [said] nucleic acid of said at least one non-target species, wherein said variable region is present in an rRNA sequence, or a DNA sequence encoding for said rRNA sequence, in a location corresponding to a target region selected from the group consisting of:

bases 65-108 of *E. coli* 5S rRNA or the encoding DNA;

bases 60-105 of *E. coli* 16S rRNA or the encoding DNA;

bases 120-150 of *E. coli* 16S rRNA or the encoding DNA;

bases 170-230 of *E. coli* 16S rRNA or the encoding DNA;

bases 405-490 of *E. coli* 16S rRNA or the encoding DNA;

bases 600-675 of *E. coli* 16S rRNA or the encoding DNA;

bases 705-735 of *E. coli* 16S rRNA or the encoding DNA;

bases 820-870 of *E. coli* 16S rRNA or the encoding DNA;

bases 980-1060 of *E. coli* 16S rRNA or the encoding DNA;

bases 1125-1155 of *E. coli* 16S rRNA or the encoding DNA;

bases 1250-1290 of *E. coli* 16S rRNA or the encoding DNA;

bases 270-405 of *E. coli* 23S rRNA or the encoding DNA;

bases 535-575 of *E. coli* 23S rRNA or the encoding DNA;

bases 1150-1200 of *E. coli* 23S rRNA or the encoding DNA;

bases 1440-1600 of *E. coli* 23S rRNA or the encoding DNA;

bases 1710-1750 of *E. coli* 23S rRNA or the encoding DNA; and

bases 2190-2330 of *E. coli* 23S rRNA or the encoding DNA[;],

provided that if said target regions is present in a location corresponding to bases 1250-1290 of *E. coli* 16S rRNA, or the encoding DNA, then said one or more target species is *Mycoplasma pneumoniae*; and

b) [detecting the presence of] determining whether a nucleic acid complex comprising said oligonucleotide probe has formed under conditions of high stringency as an indication that at least one member of said one or more target species [may be] is present in said sample, wherein [under said conditions] said oligonucleotide probe does not form a detectable duplex with [said] nucleic acid of said at least one non-target species under said conditions.

490. (Twice Amended) A method [of detecting] for determining whether one or more non-viral target [non-viral] species may be present in a sample, said method comprising the steps of:

a) contacting said sample with hybridization assay means for detecting [if] the presence of a nucleic acid variable region characteristic of nucleic acid of two or more non-viral target species belonging to a first genus, at least one of which is said one or more target species, wherein said means distinguishes said [nucleic acid] variable region [characteristic of said two or more non-viral target species] from nucleic acid of at least one non-target species belonging to a second genus which is different from said first genus, wherein said variable region is present in an rRNA sequence, or a DNA sequence encoding for said rRNA sequence, in a location corresponding to a target region selected from the group consisting of:

bases 65-108 of *E. coli* 5S rRNA or the encoding DNA;

bases 60-105 of *E. coli* 16S rRNA or the encoding DNA;

bases 120-150 of *E. coli* 16S rRNA or the encoding DNA;

bases 170-230 of *E. coli* 16S rRNA or the encoding DNA;

bases 405-490 of *E. coli* 16S rRNA or the encoding DNA;

bases 600-675 of *E. coli* 16S rRNA or the encoding DNA;

bases 705-735 of *E. coli* 16S rRNA or the encoding DNA;

bases 820-870 of *E. coli* 16S rRNA or the encoding DNA;
bases 980-1060 of *E. coli* 16S rRNA or the encoding DNA;
bases 1125-1155 of *E. coli* 16S rRNA or the encoding DNA;
bases 270-405 of *E. coli* 23S rRNA or the encoding DNA;
bases 535-575 of *E. coli* 23S rRNA or the encoding DNA;
bases 1150-1200 of *E. coli* 23S rRNA or the encoding DNA;
bases 1440-1600 of *E. coli* 23S rRNA or the encoding DNA;
bases 1710-1750 of *E. coli* 23S rRNA or the encoding DNA; and
bases 2190-2330 of *E. coli* 23S rRNA or the encoding DNA; and

b) determining [if] whether said means [detects] has detected the presence of said [nucleic acid] variable region [characteristic of said two or more non-viral target species belonging to said first genus] as an indication that at least one member of said one or more target species [may be] is present in said sample.

491. (Twice Amended) A method for [detecting] determining whether one or more non-viral target species may be present in a sample, said method comprising the steps of:

a) contacting said sample with an oligonucleotide probe able to distinguish nucleic acid of two or more non-viral target species belonging to a first genus, at least one of which is said one or more target species, from nucleic acid of at least one non-viral non-target species belonging to a second genus, wherein a duplex formed between said oligonucleotide probe and a variable region present in nucleic acid [in] of each of said two or more target species has a higher T_m than a duplex formed between said oligonucleotide probe and said variable region present in [said] nucleic acid of said at least one non-target species, wherein said variable region is present in an rRNA sequence, or a DNA sequence encoding for said rRNA sequence, in a location corresponding to a target region selected from the group consisting of :

E2

bases 65-108 of *E. coli* 5S rRNA or the encoding DNA;
bases 60-105 of *E. coli* 16S rRNA or the encoding DNA;

bases 120-150 of *E. coli* 16S rRNA or the encoding DNA;
bases 170-230 of *E. coli* 16S rRNA or the encoding DNA;
bases 405-490 of *E. coli* 16S rRNA or the encoding DNA;
bases 600-675 of *E. coli* 16S rRNA or the encoding DNA;
bases 705-735 of *E. coli* 16S rRNA or the encoding DNA;
bases 820-870 of *E. coli* 16S rRNA or the encoding DNA;
bases 980-1060 of *E. coli* 16S rRNA or the encoding DNA;
bases 1125-1155 of *E. coli* 16S rRNA or the encoding DNA;
bases 270-405 of *E. coli* 23S rRNA or the encoding DNA;
bases 535-575 of *E. coli* 23S rRNA or the encoding DNA;
bases 1150-1200 of *E. coli* 23S rRNA or the encoding DNA;
bases 1440-1600 of *E. coli* 23S rRNA or the encoding DNA;
bases 1710-1750 of *E. coli* 23S rRNA or the encoding DNA; and
bases 2190-2330 of *E. coli* 23S rRNA or the encoding DNA; and

b) [detecting the presence of] determining whether a nucleic acid complex comprising said oligonucleotide probe has formed under conditions of high stringency as an indication that at least one member of said one or more target species [may be] is present in said sample, wherein [under said conditions] said oligonucleotide probe does not form a detectable duplex with [said] nucleic acid of said at least one non-target species under said conditions.

* * * * *

Remarks

The present invention relates to Applicants' discovery that highly conserved ribosomal RNA ("rRNA") molecules (and the encoding DNA) contain specific regions of variability which can be exploited in designing probes which are capable of distinguishing between organisms. Thus, rather than being distributed randomly across entire rRNA molecules, Applicants discovered